

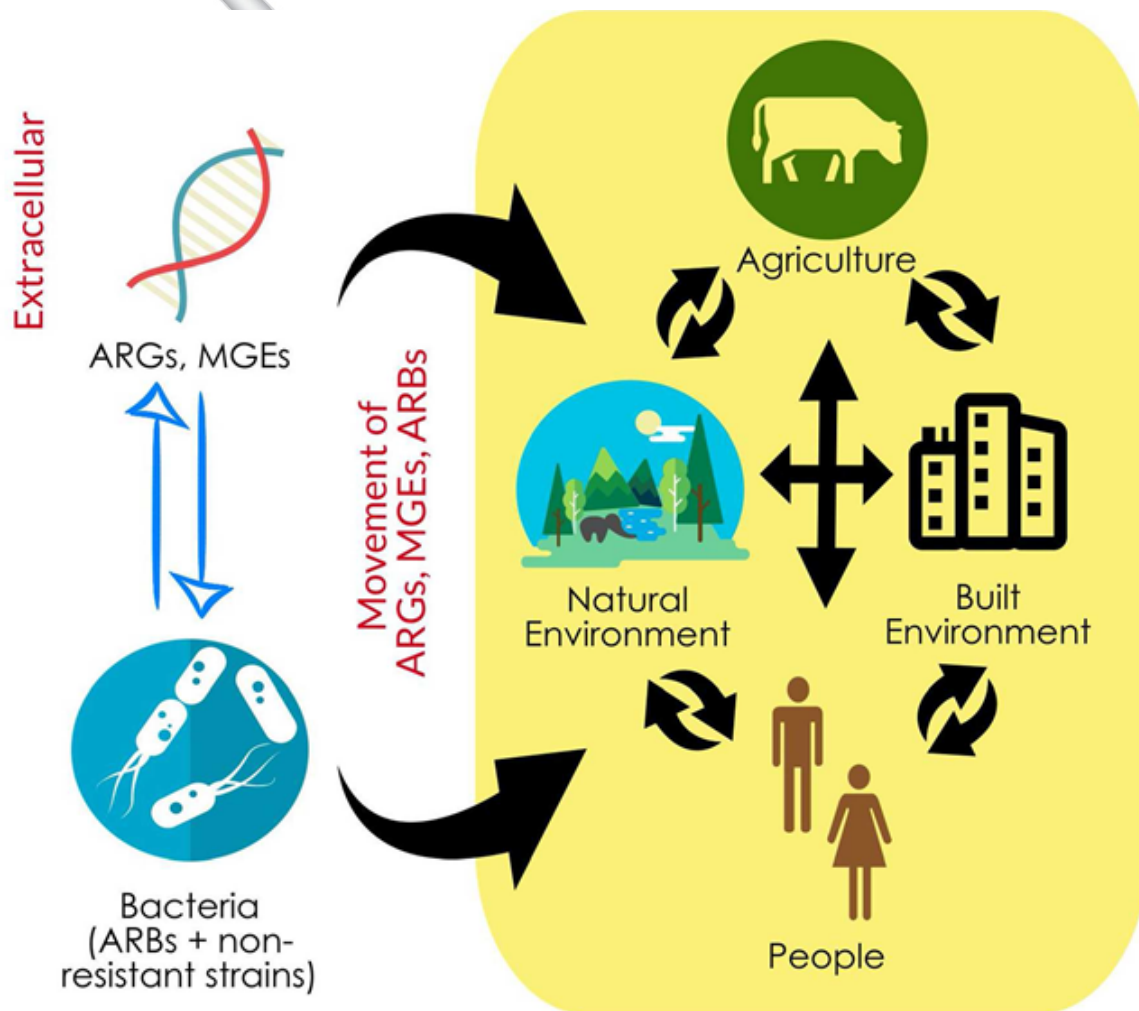


The Environmental Component of Antimicrobial Resistance: *General Background and USEPA Research*

*Jay I. Garland Ph.D
Office of Research & Development USEPA
Garland.jay@epa.gov*

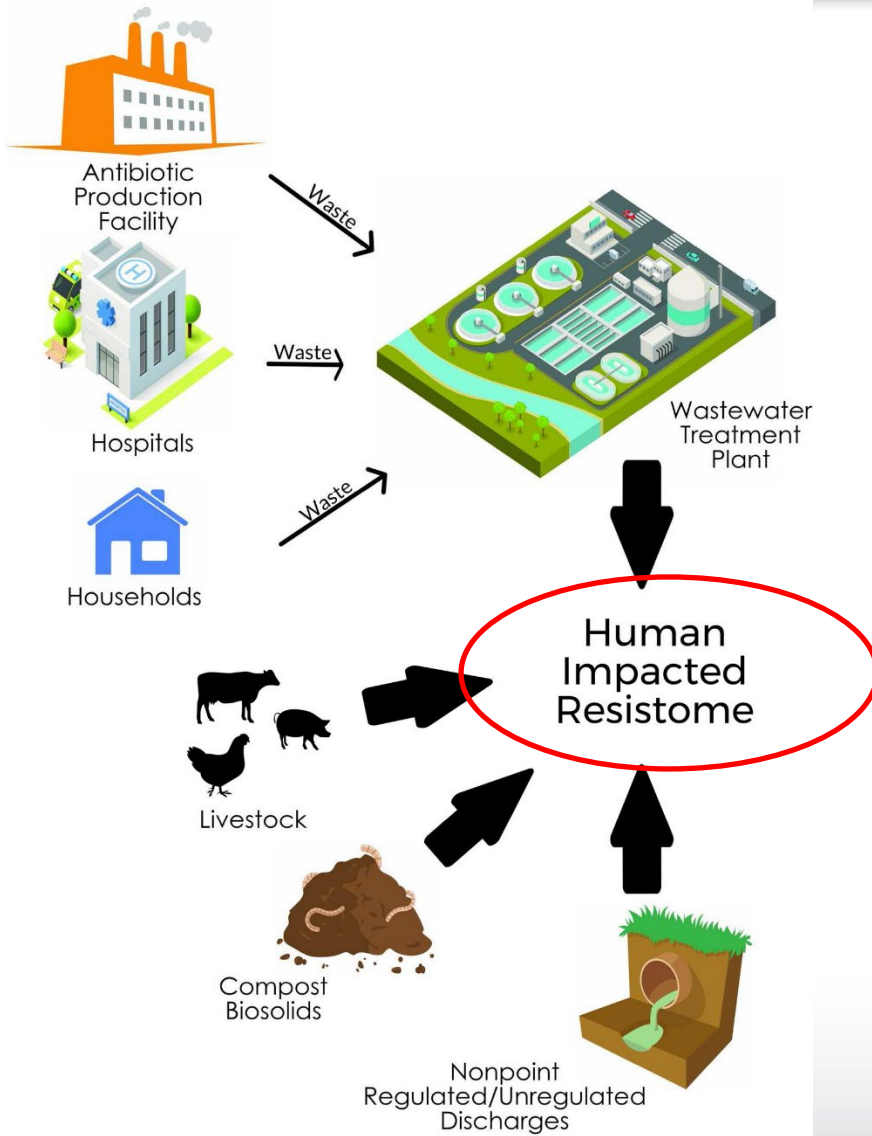
Health Burden

- Antibiotic resistance in pathogenic bacteria is a serious human-health concern
- Each year in the US:
 - 2 million people become infected with antibiotic resistant bacteria
 - 23,000 die as a direct result of infections
 - US health care system costs: \$21 to \$34 billion
 - 8 million additional hospital days





Antibiotic Resistance as an Environmental Contaminant



Natural or baseline resistome in the environment

But clear evidence for enhanced resistome due to human impacts

15 fold increase in archived Dutch soils (Knapp et al. EST 2010)

Elevated levels in areas with intensive antibiotic use (Li et al. ISME Journal 2015)

Vikesland et al. 2017. EST

Initiatives for Addressing Antibiotic Resistance in the Environment: *Current Situation and Challenges*

- Group of 40 international experts in the field of AMR drafted white paper and met to discuss at International forum in Vancouver April 2018
 - Sponsored by US CDC, UK Science & Innovation Network, and Wellcome Trust
- Key summary points & actions to address knowledge gaps in 3 areas:
 - **Human & Animal Contamination**
 - **Antimicrobial Manufacturing Wastes**
 - **Antimicrobials used as Crop Pesticides**

Human & Animal Waste (Summary)

- The connection between waste, antimicrobials, and resistant microbes in the environment, and its impact on human health, is not well understood
- Human
 - Poor global sanitation is a significant target for mitigation.
 - Release of resistant microbes from wastewater treatment systems does occur, and
 - Environmental exposures occur (e.g, MRSA infections in swimmers, greater colonization resistant *E. Coli* in surfers, MRSA linked to proximity to farms & manure application)
- Animal Farms
 - Impacts of both animal and human waste (biosolids) on the resistome in soils
 - Contamination of nearby surface and groundwaters with resistant organisms is possible
- Aquaculture
 - Large uncertainties and variation (e.g., 600-fold in Norwegian vs Chilean salmon) in antibiotic use
 - Resistance in ornamental fish can be high, and some limited reports of linkage between human infections and ornamental fish exposure

Human & Animal Waste (Summary) (cont'd)

- Methods
 - Need for standardized approaches for qPCR and metagenomic approaches, including reference materials
 - qPCR approaches are readily standardized (e.g., clinical practices) but challenges due to varying matrices and selection of gene targets
 - Need to incorporate culture based approaches, particularly for attribution/epidemiology

Human & Animal Waste (*Addressing Gaps*)

- **Assessing Environmental Waters**
 - Geospatial distribution of resistant microbes in environmental waters needed to better understand risk
 - Focused studies to understand drivers for occurrence (i.e., sources) & selective pressures for amplification and transmission
 - Evaluate sampling strategies/testing methods to identify & standardize best practices
- **Assessing & Improving Sanitation & Wastewater Treatment**
 - Improve global sanitation by identifying efficient, affordable waste processing
 - Evaluate need for on-site pretreatment from high strength facilities (e.g., hospitals)
 - Evaluate the effectiveness of existing wastewater treatment process & identify factors resulting in inefficiencies or failures
- **Assessing the Environment Related to Agriculture**
 - Identify and develop alternatives to antimicrobials
 - Evaluate methods for safe reuse of animal and human waste as fertilizer

Antimicrobial Manufacturing Waste

- No requirement to report discharge levels, little voluntary disclosure
 - Asia is the world's main producer
- Production facility wastes can result in much higher contamination of the environment than animal/human waste discharges (mg/L vs ng/L)
- Internationally recognized wastewater limits and standard method for analysis needed

Antimicrobials as Crop Pesticides

- Greater transparency & exchange of information on both the rate of use and the efficacy of treatment to guide best management, including research
- Identify and develop appropriate strategy for monitoring field and surrounding environment when medically relevant antimicrobials are used
- Bacterial control of some concern due to use of medically relevant antimicrobials (tetracycline, aminoglycosides)
 - But use is relatively targeted in space & time
- Fungicides are of greater concern, particularly azoles due to increasing use (5-fold in last 2 decades) to address emerging diseases

Larson et al. 2018. ***Critical Knowledge Gaps and Research needs related to the environmental dimensions of antibiotic resistance.***

Environment International 117, 132-138

Relative Contributions of
Different Sources

Role of Environment on
Evolution of Resistance

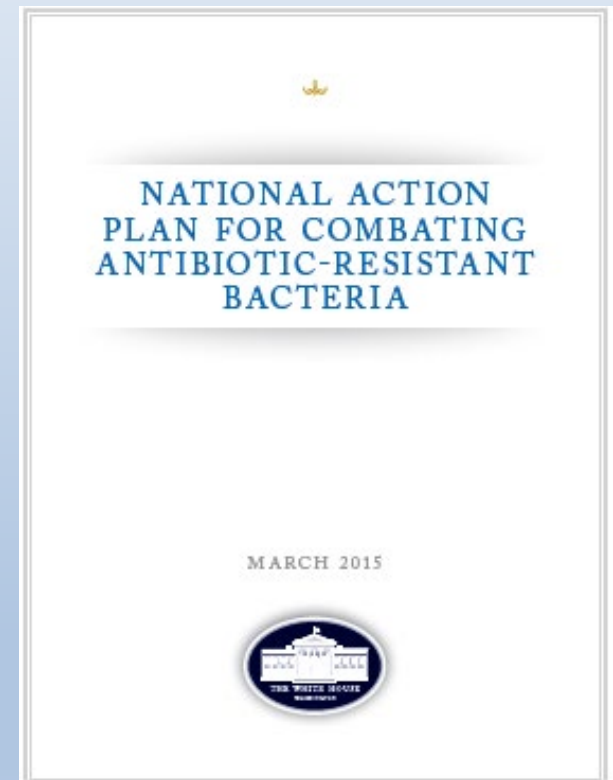
Human/Animal Health Impacts from
Environmental Exposures

Efficacy and Feasibility of
Interventions

The U.S. National Action Plan for Combating Antimicrobial-Resistant Bacteria (CARB)

Five Major Goals

1. Slow the emergence of resistant bacteria and prevent the spread of resistant infections.
2. Strengthen national one-health surveillance efforts to combat resistance.
3. Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria.
4. Accelerate basic and applied research and development for new antibiotics, other therapeutics, and vaccines.
5. Improve international collaboration and capacities for antibiotic-resistance prevention, surveillance, control, and antibiotic research and development.



Initiatives for Addressing Antibiotic Resistance in the Environment: *Current Situation and Challenges*

<https://wellcome.org/sites/default/files/antimicrobial-resistance-environment-report.pdf> (2018)

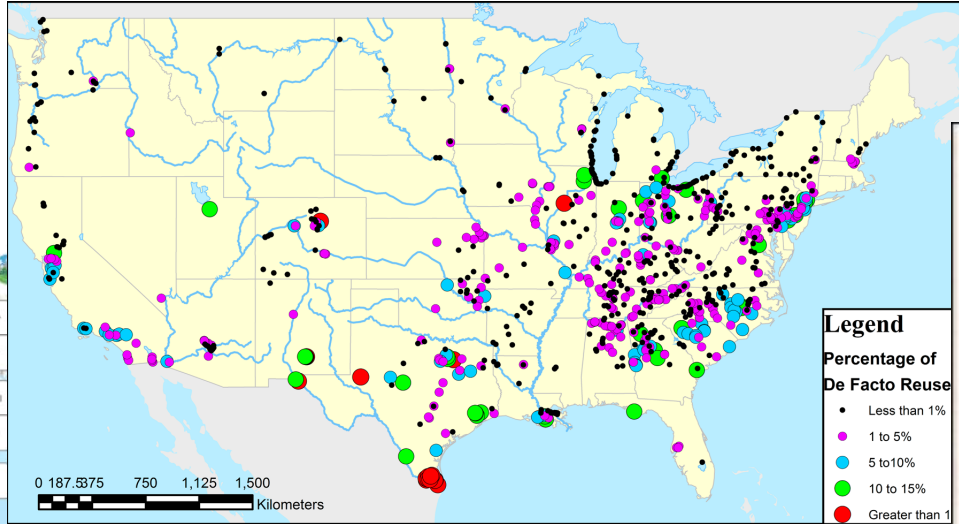
- **Environmental waters one of the areas in the report**
 - Geospatial distribution of resistance to inform risk
 - Sources & selective pressures for amplification/transmission
 - Define & standardize sampling/analysis methods

“Following the NARMS Review Subcommittee recommendations to incorporate the three major domains of the One Health model (humans, animals, environment), an important theme of this strategic plan is the expansion of testing to examine resistance in animal pathogens and the environment. For environmental monitoring, what constitutes the best sampling points will be refined over time. Surface waters as confluence points of ecosystems differentially affected by built environments is a starting point.”

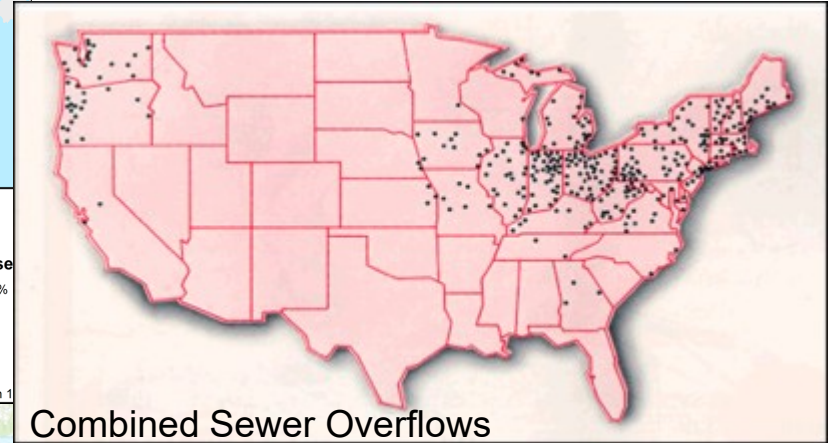
NARMS Strategic Plan 2020-2025

Why Water?

Rice J. and P. Westerhoff. 2015. Spatial and temporal variation in de facto Wastewater reuse in drinking water systems across the USA ES&T 49, 982



Human Wastewater



Combined Sewer Overflows

Animal Manure

<https://enviroatlas.epa.gov/enviroatlas/DataFactSheets/pdf/ESN/Manureapplication.pdf>

Multiple Inputs to Watersheds

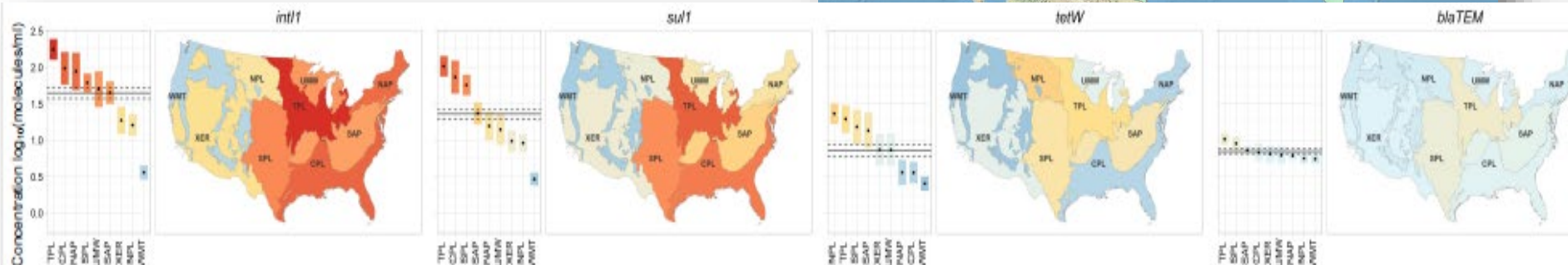
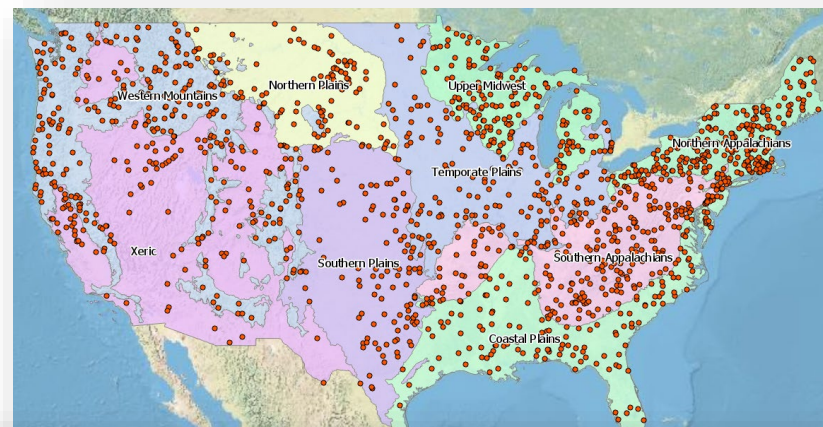
Surface Water AMR Monitoring (SWAM) *Objectives*

- A pilot environmental effort within a One Health focused NARMS
- Develop a national-scale, quantitative assessment of AMR within surface water:
 - A. Standardized measure (and library of samples) to monitor trends as part of NARMS
 - B. Input to models of AMR risks for various end uses of water (recreational, drinking, agricultural, water reuse).
 - C. Help quantify drivers of occurrence and selective pressures for potential amplification
 - D. Identify critical control points and assess current and new mitigation strategies

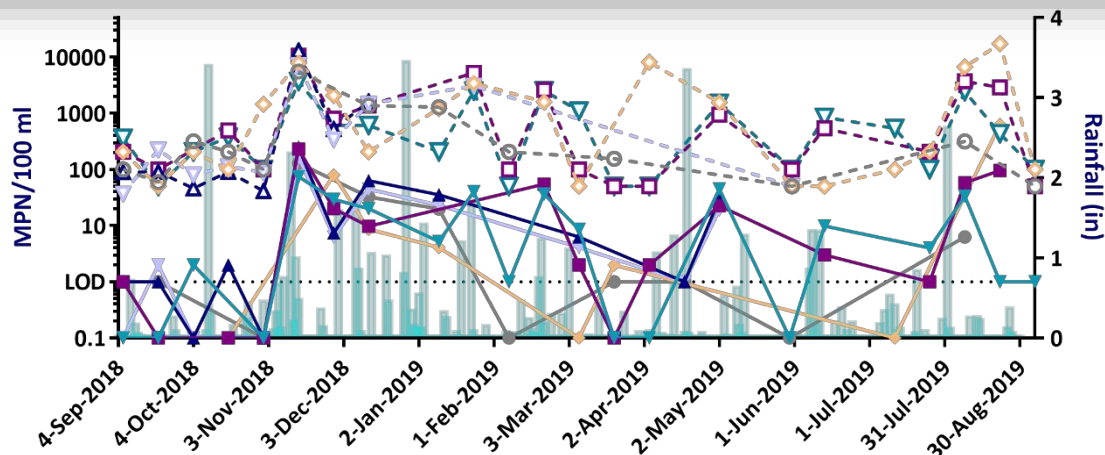
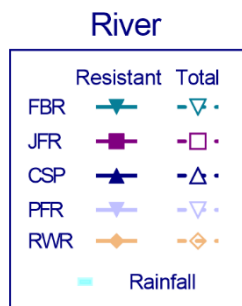
Designing the Study

Go Big and Slow?

EPA National Rivers and Streams Assessment
5 year, probabilistic survey of aquatic resource



Or Small and Fast?

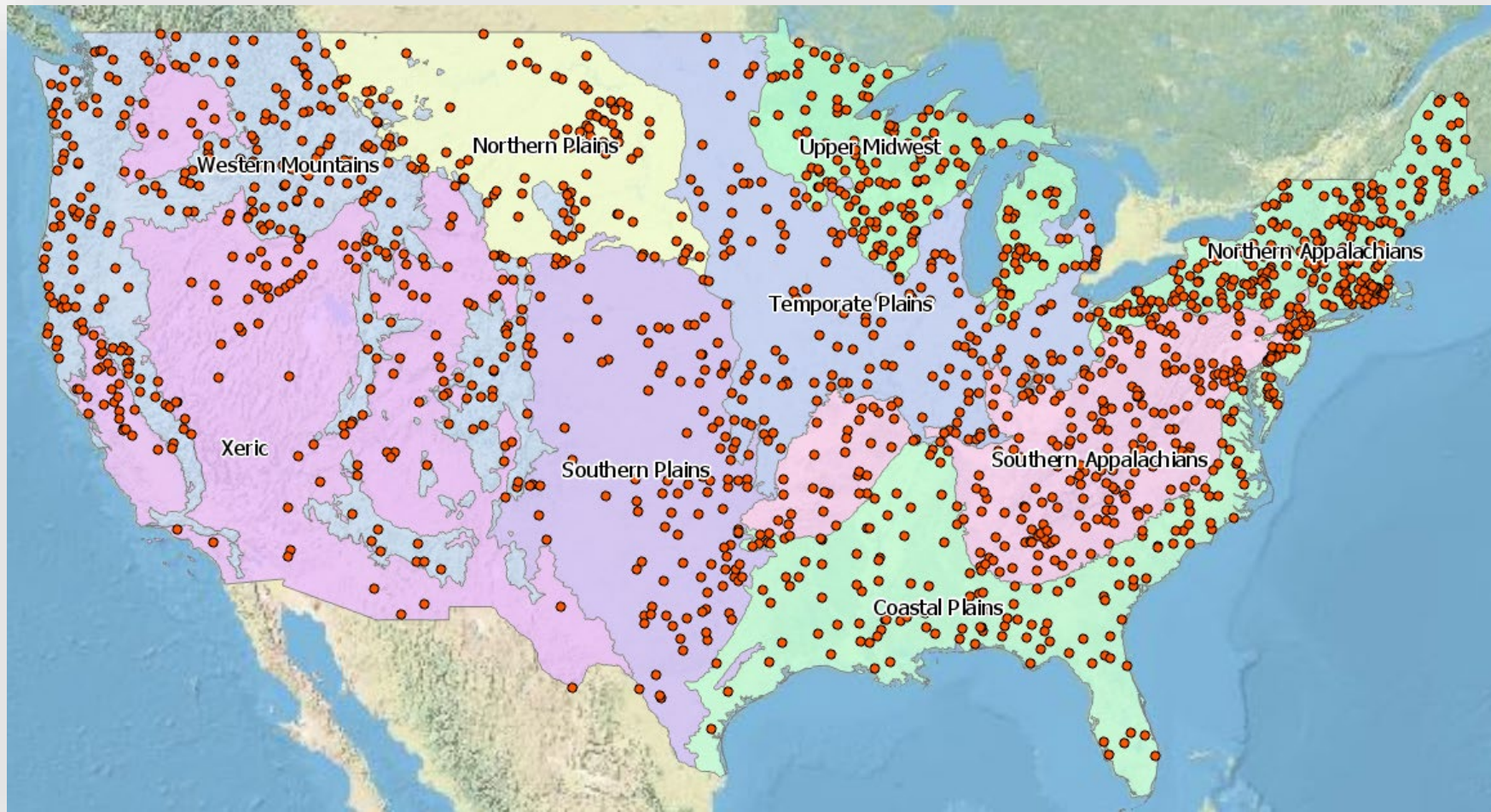


CDC Preliminary Surface Water Study in Chattahoochee River

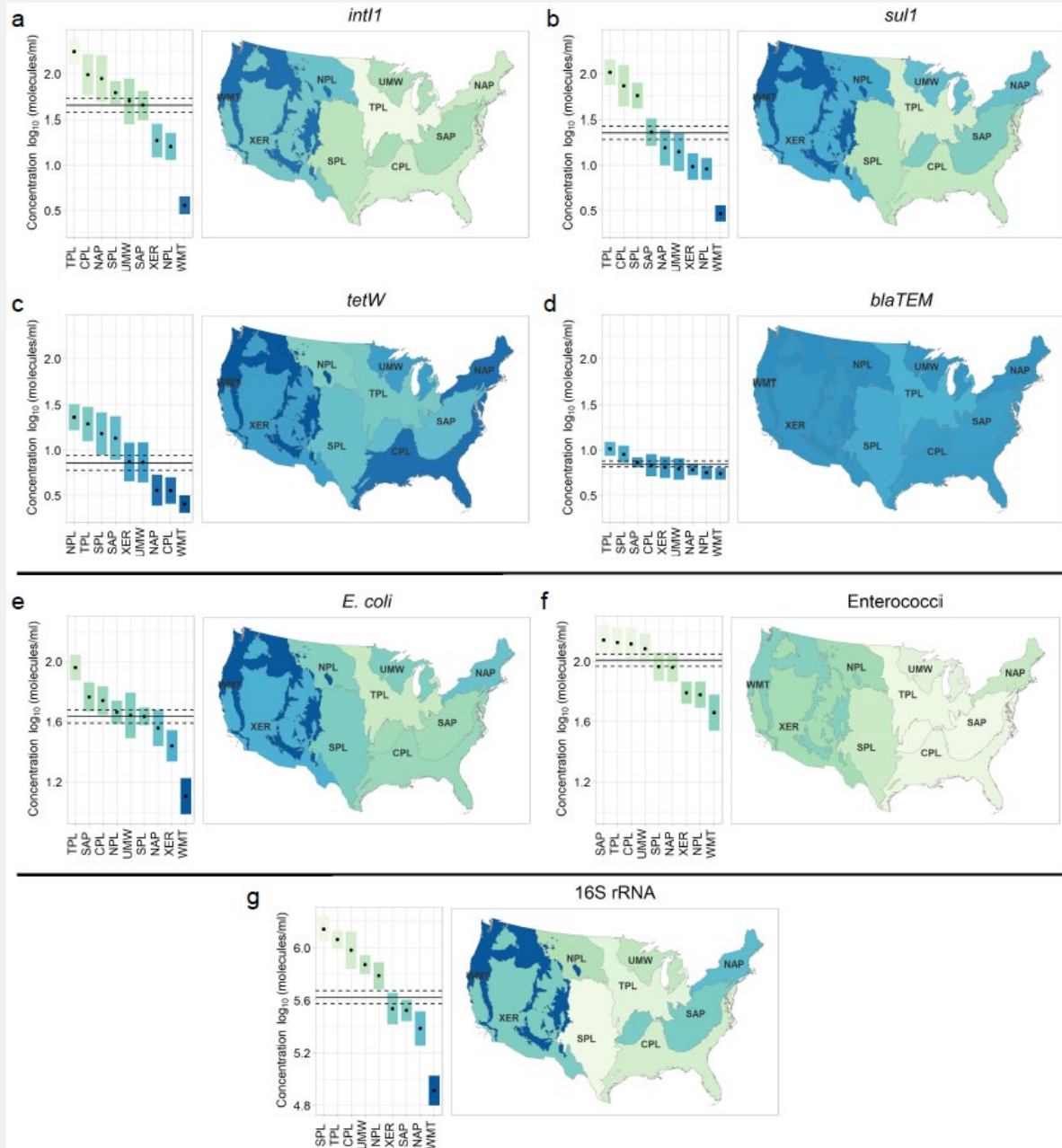
Phased design for SWAM

Phase 1	SWAM Pilot	Statistical Design Subgroup discussions	Initial testing of methodologies	FY21-1 st half FY22
Phase 2			Watershed based assessment to trial methodologies before national sampling and serve as a demonstration project for future watershed studies	Spring FY22-Spring FY23
Phase 3			Probabilistic national survey to provide statistically valid estimates of AMR status and trends in surface water, using methods tested in the other phases	Summers 2023-24,
Phase 4			Continued probabilistic national monitoring together with expanding number of (partner-led) intensive watershed studies across the country	2024+

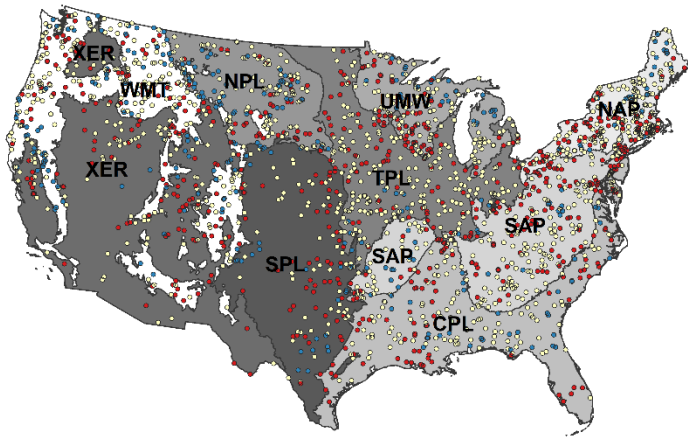
Leveraging EPA Programs



Map of National Rivers and Streams Assessment
(ECO9 Regions Shown)



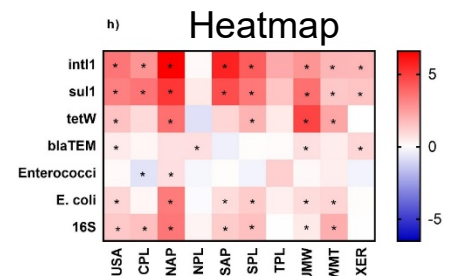
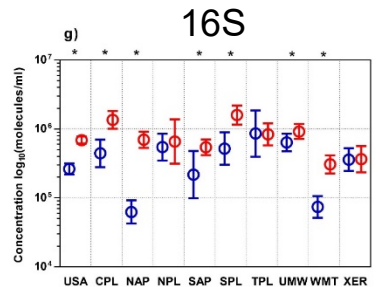
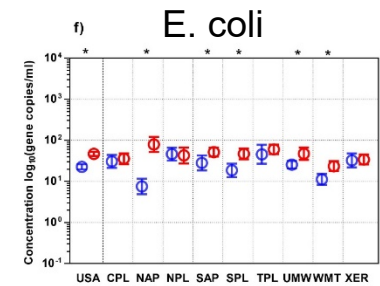
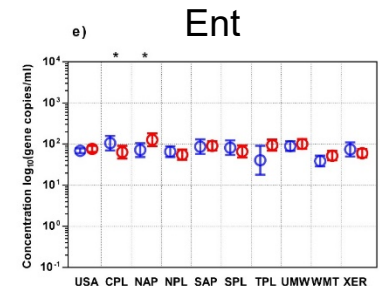
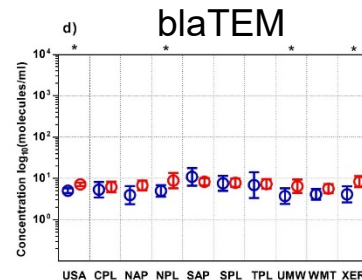
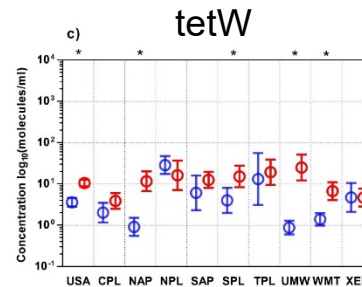
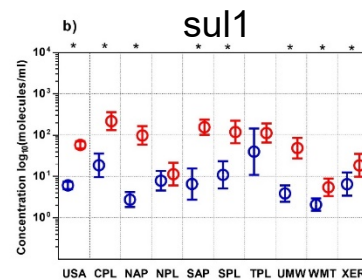
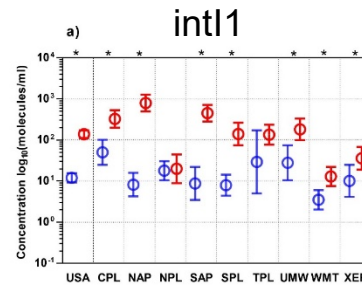
Baseline Analysis



ECO9 Regions Shown; **blue** is reference;
yellow is intermediate; **red** is impacted

Filter criteria

Total P ($\mu\text{g/L}$)
Total N ($\mu\text{g/L}$)
Cl⁻ ($\mu\text{eq/L}$)
SO₄²⁻ ($\mu\text{eq/L}$)
ANC ($\mu\text{eq/L}$), DOC
(mg/L)
Turbidity (NTU)
Riparian Disturbance
Index
% fine substrate



Watershed studies complement NRSA design

Is there temporal/seasonal variation in antimicrobial resistant bacteria and genes?

Are there environmental reservoirs of AMR?

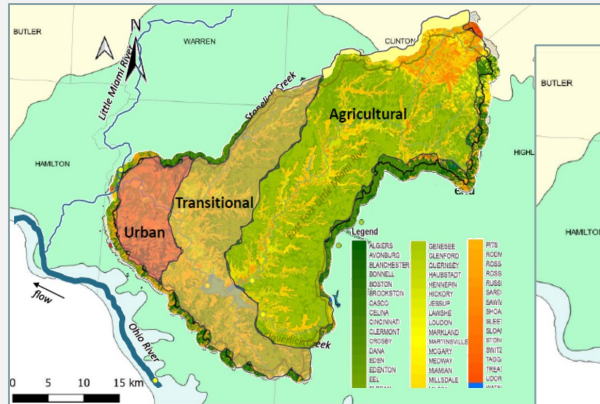
What are the relative contributions of different AMR sources (e.g., septic, WWTP, livestock, wildlife)

What are the watershed-scale drivers and attenuators of AMR?

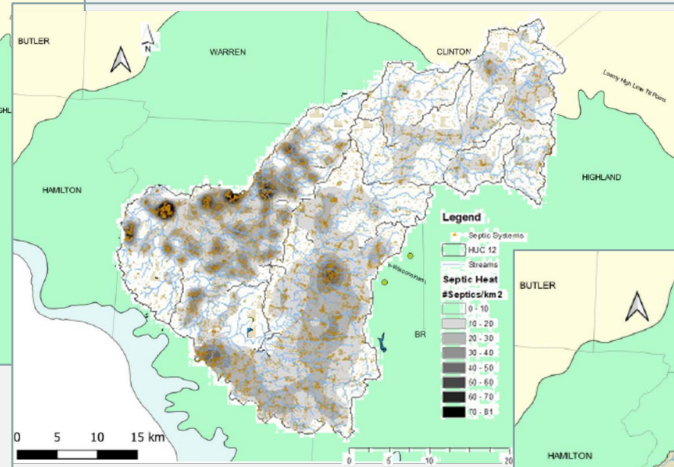
How can we mitigate AMR at local scales?



East Fork Little Miami Watershed AMR Pilot



Urban- Ag transition



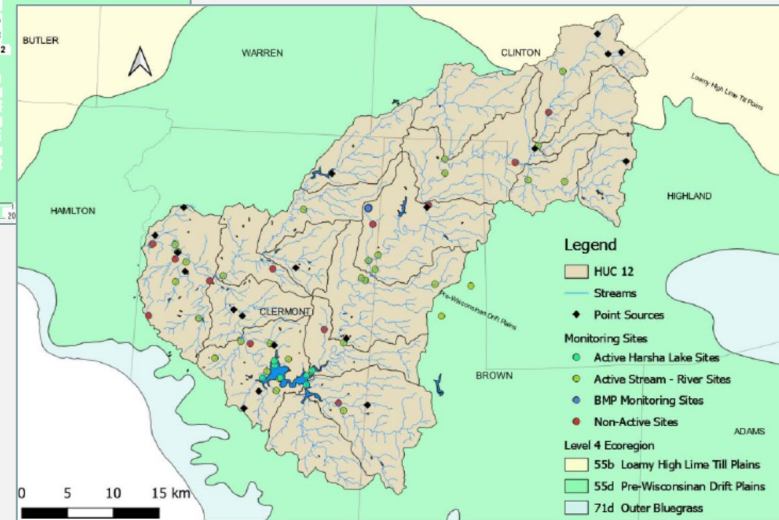
12K septic systems mapped



Will determine minimum reporting and data quality objectives for comparisons to NRSA and future watershed studies

Additional watershed studies needed:

- High livestock inputs
- Highly urbanized systems
- Regional variation



Point sources and rec waters in relation to sample sites

NRSA Sampling

- **Past AMR Work (2014/2015 & 2018/2019)**
 - Utilized 50 ml *Enterococcus* samples
Filtered on site, frozen, and shipped
 - Could only do DNA work and amount of bacteria DNA was minimal due to small volume
 - Targeted gene analysis of 5-10 genes (many non-detects)
 - Limited metagenomic work due to small amount of sample.
- **Proposed Pilot Study**
 - Collect 4 L of water in a cubitainor
 - Location in waterway to be determined
 - Keep on ice and ship overnight
 - Protocol in development over next 6 months
 - Collection of whole water samples and larger volumes allows all culture and molecular work to be performed.

Analytical Work

Culture-based and molecular-based analysis to characterize antimicrobial resistance in surface waters. Each technique has its own advantages and disadvantages.

- **Culture**

- Determine expression of resistance in bacteria
- Limited to bacteria that are culturable (Only ~1% of environmental bacteria)

- **Targeted Gene Analysis**

- Quantitative measure – Understand if resistance is elevated
- Limited to known genes
- Do not know if gene (resistance) is expressed

- **Metagenomics**

- Identify most, if not all, antimicrobial resistance genes (resistome)
- Time intensive and not quantitative
- Do not know if gene (resistance) is expressed

Analytical Targets

- **Culture**

- *Enterococci, E.coli*: Links to existing water quality methods
 - Will quantify and determine resistance to specific antibiotics
- *Salmonella*: Links to food cycle & NARMS
 - Presence/absence

- **Targeted Gene Analysis**

- Defined panel of antibiotic resistance genes important to human, animal, and environmental health, including fecal source trackers (~90-100 genes)

- **Metagenomics**

- Define environmental resistome in surface waters
- Determine new genes to quantify via targeted gene analysis

Other Activities

- Development of risk models (beginning with recreational water exposures)
- National scale wastewater monitoring
 - Probabilistic survey designs
 - Both into and out of treatment plants
- Research to inform better risk assessment for pesticide registration